

PATENT APPLICATION

**METHOD OF PREPARING BIOLOGICAL MATERIALS FOR  
CRYOPRESERVATION USING PRE-CHILLED PROTECTANT**

**ABSTRACT OF THE DISCLOSURE**

A method of preparing biological materials for cryopreservation is presented.

5 The method lessens the amount of heat released by a cryoprotectant during a latent  
heat phase by freezing the protectant, thawing the protectant, and treating biologically  
active materials with the thawed protectant. First, the protectant is frozen to induce  
an irreversible phase change, along with an irreversible release of energy. After this  
phase change has occurred, the protectant is thawed and used to treat viable cells or  
10 other biologically active material about to undergo freezing. The thawed protectant  
within the biologically active cells has a reduced endothermic reaction upon  
subsequent freezing. In one embodiment, freezing the biological material includes  
immersing the biological material in cooling fluid and circulating the cooling fluid  
past the biological material at a substantially constant, pre-determined velocity and  
15 temperature such that the biological material is vitrified, thus minimizing the  
formation of stress fractures in cell membranes. The protectant may be propylene  
glycol, glycerol, DMSO, or other suitable protectants.